Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference M29255PC	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/06861	International filing date (*day/month/year*) 18 July 2000 (18.07.00)	Priority date (*day/month/year*) 19 July 1999 (19.07.99)
International Patent Classification (IPC) or national classification and IPC C12N 15/864,		
Applicant MEDIGENE AKTIENGESELLSCHAFT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26 January 2001 (26.01.01)	11 January 2002 (11.01.2002)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP00/06861

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

☒ the international application as originally filed.

☒ the description, pages 1-41, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-26, filed with the letter of 26 January 2001 (26.01.2001),
Nos. _____, filed with the letter of _____.

☒ the drawings, sheets/fig 1/2 - 2/2, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-26	**YES**
	Claims		**NO**
Inventive step (IS)	Claims		**YES**
	Claims	1-26	**NO**
Industrial applicability (IA)	Claims	1-26	**YES**
	Claims		**NO**

2. Citations and explanations

1. Novelty

The present application meets the requirements of PCT Article 33(2) because the subject matter of Claims 1-26 is novel within the meaning of PCT Article 33.

2. Inventive step

The present application does not meet the requirements of PCT Article 33(3) because the subject matter of Claims 1-26 does not involve an inventive step within the meaning of PCT Article 33.

2.1 Claims 1-10, 12, 15-24 and 26

The present application addresses the problem of modifying the chromatographic properties of the AAV virus, in particular of a structural protein, in comparison with the wild type (page 4, paragraph 2). According to the description, the modification of the chromatographic properties preferably improves purification, in particular virus enrichment, purification to a higher degree of purity and/or more efficient purification (page 4, paragraph 3).

The solution according to Claim 1 consists in modifying the chromatographic properties of the virus by mutating its structural protein.

The solution described in Claim 1 concerns all possible mutations. However, the application does not demonstrate that all mutations can cause a modification of the chromatographic properties.

Consequently, Claim 1 does not solve the problem and cannot involve an inventive step (PCT Article 33(3)).

Claims 2-10, 12 and 15-24 do not further specify the mutations. Consequently, an inventive step cannot be recognised in Claims 2-10, 12 and 15-24 either.

2.2 Claims 11 and 25

Claims 11 and 25 concern mutations or the insertion of various peptide fragments into a structural protein, these mutations being characterised by an exact structural feature.

However, the application does not demonstrate that these modifications of the structural proteins of the AAV virus modify the chromatographic properties of the virus protein. Figures 1 and 2 cannot be compared with one another and precisely evaluated because they show different absorption scales (right-hand Y-axis). In this case, the figures cannot be exactly evaluated whether the experimental conditions were the same or not. Moreover, it is not clear whether the problem of reducing impurities has been solved thereby.

Consequently, Claims 11 and 25 do not solve the problem and an inventive step cannot be recognised (PCT Article 33).

3. Industrial applicability

The present application meets the requirements of PCT

Article 33(4) because the subject matter of Claims 1-26 is industrially applicable within the meaning of PCT Article 33.

PATENT CLAIMS

1. A structural protein of adeno-associated virus (AAV), characterized in that the structural protein comprises at least one mutation which brings about an alteration in the chromatographic properties of the virus.

2. A structural protein as claimed in claim 1, characterized in that the alteration in the chromatographic properties makes an improvement in the purification possible, in particular a concentration of the virus, preferably of the virus particles, to higher titers, a purification to greater purity and/or a more efficient purification.

3. A structural protein as claimed in either of claims 1 or 2, characterized in that the mutation brings about a negligible reduction in the infectivity of the virus.

4. A structural protein as claimed in any of claims 1 to 3, characterized in that the mutation also brings about an increase in the infectivity of the virus.

5. A structural protein as claimed in any of claims 1 to 4, characterized in that the mutated structural protein is capable of particle formation.

6. A structural protein as claimed in any of claims 1 to 5, characterized in that the mutated structural protein increases the thermal stability.

7. A structural protein as claimed in any of claims 1 to 6, characterized in that it is selected from mutated VP1, mutated VP2 and/or mutated VP3.

8. A structural protein as claimed in any of claims 1 to 7, characterized in that it is derived from AAV1, AAV2, AAV3, AAV4, AAV5 and/or AAV6 and other AAV serotypes derived therefrom, in particular from AAV2.

9. A structural protein as claimed in any of claims 1 to 8, characterized in that the mutation is a point mutation, a mutation of more than one amino acid, one or more deletion(s), in particular one or more insertion(s) or a combination of said modifications.

10. A structural protein as claimed in any of claims 1 to 9, characterized in that amino acids of a functional sequence which are preferably suitable for affinity chromatography are inserted.

11. A structural protein as claimed in claim 10, characterized in that the inserted amino acid sequence is selected from a ligand of a receptor or the receptor of a ligand, an antibody or part of an antibody, in particular an antibody epitope, an antigen or antigen epitope, a hormone, a hormo-receptor, an enzyme, an enzyme substrate, a lectin, sugar-bearing amino acids, in particular from a histidine-rich peptide (His tag), a multiply charged peptide, glutathione S-transferase (GST tag), an F_c part of an antibody, an immunoglobulin-binding domain, for example protein A or protein G or a part thereof, a lecitin, a nucleic acid binding site, a heparin binding site, a specific ligand, a specific receptor, an integrin, a cytokine or a receptor binding domain of a cytokine, integrin or growth factor, single-chain antibodies which bind to a cell surface receptor, an antibody against cell surface structures, an epitope and/or an antibody-binding structure.

12. A structural protein as claimed in either of claims 10 or 11, characterized in that a peptide which has the sequence QAGTFALRGDNPQG is inserted.

13. A structural protein as claimed in any of claims 1 to 12, characterized in that the structural protein comprises at least one other mutation.

14. A structural protein as claimed in claim 13, characterized in that the other mutation(s) brings about an alteration in the infectivity of the virus.

15. A structural protein as claimed in either of claims 13 or 14, characterized in that the other mutation(s) brings about a reduction in the antigenicity of the virus.

16. A structural protein as claimed in any of claims 13 to 15, characterized in that the other mutation(s) is/are one or more deletion(s), one or more insertion(s) or a combination of said modifications.

17. A structural protein as claimed in any of claims 13 to 16, characterized in that the insertion is a cell membrane receptor ligand, a Rep protein or peptide, an immunosuppressive protein or peptide and/or a protein or peptide with a signal for double strand synthesis of the foreign gene.

18. A structural protein as claimed in any of claims 13 to 17, characterized in that the insertion is selected from an integrin, a cytokine or a receptor binding domain of a cytokine, integrin or growth factor, single-chain antibodies which bind to a cell surface receptor, an antibody

against cell surface structures, an antibody-binding structure or an epitope.

19. A structural protein as claimed in any of claims 1 to 18, characterized in that the mutation(s) is/are located on the virus surface.

20. A structural protein as claimed in any of claims 1 to 19, characterized in that the mutation(s) is/are located at the N terminus of the structural protein.

21. A structural protein as claimed in any of claims 1 to 20, characterized in that the mutation(s) is/are brought about by one or more insertions in the XhoI cleavage site of the VP1-encoding nucleic acid.

22. A structural protein as claimed in any of claims 1 to 21, characterized in that the mutation(s) is/are brought about by one or more insertions in the BsrBI cleavage site of the VP1-encoding nucleic acid.

23. A structural protein as claimed in any of claims 1 to 22, characterized in that the mutation(s) is/are brought about by one or more deletions between the BsrBI-HindII cleavage sites of the VP1-encoding nucleic acid and one or more insertions.

24. A structural protein as claimed in any of claims 1 to 23, characterized in that the mutation(s) is/are brought about by one or more deletions between the XhoI-XhoI cleavage sites of the VP1-encoding nucleic acid.

25. A structural protein as claimed in any of claims 1 to 24, characterized in that the mutation(s)

is/are brought about by one or more deletions between the BsrBI-HindII cleavage sites of the VP1-encoding nucleic acid.

26. A structural protein as claimed in any of claims 1 to 20, characterized in that one or more insertions in VP3 is/are located before and/or after at least one amino acid in the sequence selected from YKQIS SQSGA, YLTLN NGSQA, YYLSR TNTPS, EEKFF PQSGV, NPVAT EQYGS, LQRGN RQAAT, NVDFT VDTNG.

27. A structural protein as claimed in any of claims 1 to 26 in the form of an AAV particle, in particular in the form of an AAV capsid.

28. A nucleic acid coding for a structural protein as claimed in any of claims 1 to 27.

29. A cell comprising a nucleic acid as claimed in claim 28.

30. A method for producing a structural protein as claimed in any of claims 1 to 27, characterized in that a cell as claimed in claim 29 is cultivated and, where appropriate, the expressed structural protein is isolated.

31. A pharmaceutical comprising a structural protein as claimed in any of claims 1 to 27, a nucleic acid as claimed in claim 28 and/or a cell as claimed in claim 29 and/or where appropriate excipients and/or additives.

32. The use of a structural protein as claimed in any of claims 1 to 27, of a nucleic acid as claimed in claim 28 or of a cell as claimed in claim 29 for the purification of AAV and AAV particles, for altering the tropism of AAV, for altering the

antigenicity of AAV, for transforming a cell, for genomic targeting, for diagnosis, for activity investigations and/or for gene therapy.